The Electrical Potential Profile of the Isolated Toad Bladder

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ABSTRACT The electrical potential profile of the isolated toad bladder was examined in the spontaneously active, chronically short-circuited, and intermittently short-circuited states by means of glass micropipettes. The position of the micropipette tip within the bladder was evaluated by measuring the D.C. resistance between the micropipette tip and the reference electrode on the serosal side of the bladder. In the spontaneously active state, with concentrations of sodium in the mucosal solution ranging from less than 1 to 114 meq per liter, the potential profile consisted in the majority of impalements of two steps, each positive to the mucosal solution. A minority of impalements showed more than two potential steps, each positive to the mucosal solution. In the short-circuited state, the interior of the bladder was found to be negative to the bathing solution by approximately 5 mv. The results are interpreted as showing a potential step at the two surfaces of the epithelial cell layer of the toad bladder. In the spontaneously active state the potential change at the mucosal boundary is of the wrong polarity to bring about net sodium entry; the small electrical driving force across the mucosal surface which is present in the short-circuited state may contribute to the net entry of sodium from mucosal solutions with low sodium concentration.

INTRODUCTION

Previous studies have demonstrated that the urinary bladder of the toad is capable of active transport of sodium from its urinary or mucosal side to its body or serosal side (1). Since the bladder consists essentially of a single layer of epithelial cells supported on a thin connective tissue stroma, this process can be resolved into two components: the uptake of sodium from the medium at the mucosal boundary, and the extrusion of sodium from the cell interior into the medium on the serosal side. This and the following paper are concerned with the first of these processes, the forces responsible for the net entry of sodium from the mucosal medium into the epithelial cell layer of the bladder.

Three possibilities can be suggested to account for the fact that net entry of sodium across the mucosal boundary does occur. First, the interior of the epithelial cell may be electrically negative to the mucosal medium. Second, a concentration gradient for sodium may be present. Third, an inward directed active transport step for sodium may take place in the mucosal surface. The present study is concerned with the description of the electrical potential profile of the toad bladder. The results indicate that electrical driving forces cannot be responsible for the net mucosal entry of sodium in the spontaneously active bladder, and make a small contribution to the driving force for sodium entry in the short-circuited bladder.

METHODS

The studies were performed on the isolated urinary bladder of the toad, *Bufo marinus*. Male or female animals were selected, pithed, the bladder removed, everted, and mounted with the mucosal side out on a cylinder of frog Ringer's solution containing 2 per cent agar supported on a lucite frame. The assembly was then suspended in the mucosal bathing medium on the stage of a dissecting microscope in such a way that the mucosal and serosal solutions were separated by the bladder wall. The Ringer-agar cylinder contained the end of the Ringer-agar bridge which constituted the reference potential point, and a silver-silver chloride electrode. This electrode was connected through an external voltage divider to another silver-silver chloride electrode, in the mucosal medium, arranged as a loop around the bladder at a distance of 2 cm from it. By means of this external circuit and the two silver-silver chloride electrodes, the spontaneous transbladder potential could be nullified by the passage of a current through the solutions. The current required to do this, which is equivalent to the active transport of sodium under these conditions, could be monitored by a Weston D.C. microammeter as described in previous publications from this laboratory (1).

A. Solutions

The frog Ringer's solution used in these experiments had the following composition: NaCl 111.1 mM, KCl 3.5 mM, $NaHCO_3$ 2.38 mM, $CaCl_2$ 0.89 mM. The agar-supporting cylinder to which the serosal surface of the bladder was exposed consisted of the above solution, 2 gm of bacto-agar (Difco Laboratories, Detroit, Michigan) per 100 ml of Ringer's, and atropine sulfate U.S.P. to give a final concentration of atropine of 1 mM. Control experiments using a chamber apparatus similar to that of Ussing and Zerahn (2) demonstrated that this concentration of atropine sulfate in the serosal bathing medium did not affect adversely the transbladder potential, short-circuit current, or viability of the bladder.

The sodium concentration in the mucosal bathing medium was adjusted by dilution of the standard frog Ringer's with the required volume of a solution identical to the Ringer's except for the substitution of choline bicarbonate and chloride for the sodium bicarbonate and chloride.

B. Micropipettes

Ling-Gerard glass micropipettes (3) were pulled from 2 mm O.D. borosilicate glass on a model M-1 micropipette puller (Industrial Science Associates, Ridgewood, New York). The pipettes were filled immediately with filtered 3 M KCl (4) by boiling at reduced pressure, were then stored in 3 M KCl, and used within 24 hours. The micropipette holder was supported in a micromanipulator, the excursions of which could be measured on a gauge to a precision of 1 micron. The micropipette resistance and tip potential in Ringer solution were checked immediately before and after each impalement by the method of Adrian (5) and the observation discarded if the micropipette resistance fell below 5 megohms, if the tip potential was more negative than −5 mv, or if the potential recorded in the bladder was not stable for at least 5 seconds.

Because the tip of the micropipette could not be resolved by the dissecting microscope and the position of the bladder could not be fixed rigidly in space, it was desirable to have a method of determining the relative position of the pipette tip and the bladder epithelial cell at the time the observation of the potential was made. Attempts to introduce a marking material from the micropipette into the cell by means of electrophoresis or positive pressure did not give satisfactory results and an electrical method of getting the same information was finally adopted. With the micropipette tip in the mucosal medium and the reference electrode on the serosal side, the current necessary to nullify the spontaneous transbladder potential was introduced from the external voltage divider. This short-circuit current was then switched on and off at a rate of 9 cycles per minute while the electrode was advanced in steps of from 3 to 8 microns through the bladder. The low switching rate minimized current effects due to membrane capacitance, and the envelope of the resulting potentials gave values for the electrical potential as a function of distance through the bladder in the spontaneously active and short-circuited states, and an approximation of the relative D.C. resistance of the portion of the bladder between the micropipette tip and the reference electrode in the serosal medium.

C. Recording

A chlorided silver wire made contact with the 3 M KCl in the micropipette, and was connected to one input terminal of a model 200 B D.C. vacuum tube voltmeter (Keithley Instruments, Cleveland). On repeated determinations the grid current of the input stage was never found to be more than 5×10^{-13} A. The Ringer-agar bridge from the serosal surface of the bladder led to a chlorided silver wire in a solution of 3 M KCl. A voltage calibrator containing a mercury battery as the reference potential was connected between the silver-silver chloride electrode and the second input terminal of the vacuum tube voltmeter. The output of the voltmeter was fed into a series 153X17 electronik recorder (Minneapolis-Honeywell Regulator Co., Philadelphia). The limiting frequency response of the circuit was determined by the recorder, and, at a switching rate of 9 cycles per minute, the record showed 98.5 per cent of full scale deflection for the same potential step at zero frequency.

D. Artifacts

Adrian (5) has shown that the potentials registered by high resistance glass micropipettes are not independent of the composition of the solution in which the pipette tip is placed. In the present study, the tip potentials measured in ordinary Ringer's solution were compared with those measured in a mixture of ordinary and potassium Ringer's solution, the cationic composition of which roughly approximated that in the non-inulin space water of the toad bladder. The difference in tip potentials of the micropipettes in these two solutions averaged 2.5 mv, being more negative in the high potassium solution, and was nearly independent of micropipette resistance or tip potential in Ringer's solution within the range of these values in pipettes which would have been suitable for experimental use.

A more troublesome artifact was that associated with mechanical distortion of the micropipette during penetration of the tissue. The scattered smooth muscle bundles on the serosal surface of the bladder are spontaneously active both *in vivo* and *in vitro*, giving rise to periodic shifts in the position of a given section of the bladder surface. The waves of contraction were easily seen under the dissecting microscope and the passage of one past the site of an impalement was associated with wide swings in the recorded potential, often the breaking of the micropipette tip, and, very probably, substantial injury to the cell membrane around the site of impalement. The frequency of occurrence of these contractions was reduced but not eliminated by the addition of 1 mM atropine sulfate to the serosal medium.

Because of the possibility that more stable shifts in recorded potential might occur as the result of mechanical distortion of the micropipette during impalement, experiments were performed on bladders soaked for 10 minutes in 0.1 N HCl. This treatment abolished all spontaneous electrical activity but left the bladder with nearly normal consistency. In a series of sixteen satisfactory impalements, the initial stable potential change on penetrating the bladder had a mean value of -1.5 mv with a range of 0 to -5 mv. This value is considered to be within the limits of experimental error and has not been used to correct the potential observations reported below.

RESULTS

A. The Serosal Limiting Membrane

Fig. 1 is a schematic representation of a cross-section of the toad bladder. The portion marked *A* is the single layer of epithelial cells which performs the active reabsorption of sodium, a characteristic function of the organ. *B* is a loose matrix of collagen fibers, smooth muscle bundles, and capillaries which serves as a mechanical support for the bladder and its blood supply, and *C* is a thin serosal limiting membrane which lines the body cavity of the toad and is reflected over the surface of the viscera. Since the position of the micropipette tip in the bladder was determined indirectly, by the fraction of the total D.C. resistance of the bladder remaining between the micropipette tip

and the reference electrode on the serosal side of the bladder, it was important to evaluate the component of the membrane resistance which was due to the serosal limiting membrane alone. Further, the value of the potential recorded within the substance of the bladder relative to the serosal reference electrode would be altered by the amount of any potential developed by the serosal limiting membrane, hence it was necessary to determine whether it was capable of generating an electrical potential.

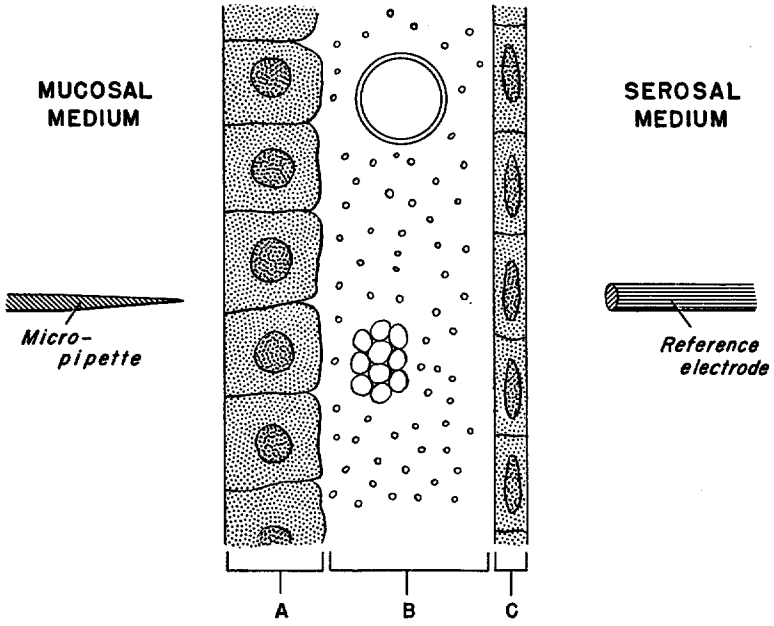

FIGURE 1. Schematic representation of a cross-section of the toad bladder. Section marked *A* is the single layer of bladder epithelial cells at the mucosal border of the membrane. *B* is a layer of loose connective tissue made up of collagen fibers, smooth muscle bundles, and capillaries. *C* is the serosal limiting membrane.

It was not possible to isolate a portion of this membrane in undamaged form from the serosal surface of the bladder itself, but dorsal and rostral to its reflection onto the bladder it lines the body cavity unattached to visceral or parietal structures. Membranes from this location in six toads were mounted in a small lucite chamber of the same general design as that used by Ussing and Zerahn (2). In contrast to the finding in toad bladders, no spontaneous transmembrane potentials were observed, and the average D.C. resistance of the serosal limiting membranes was found to be 5 per cent of that in fourteen similarly mounted toad bladders. This figure of 5 per cent of the total resistance of the bladder must necessarily be regarded as an approximation, since there was no way of comparing precisely the true areas of the different membranes within the limits of the chamber. From the data, it is

possible to exclude a contribution to the transbladder potential due to unidirectional active transport in the serosal limiting membrane. It is not possible to exclude the existence of some form of diffusion potential across the serosal limiting membrane under some conditions, but the low D.C. resistance of the serosal limiting membrane as compared to that of the toad bladder makes it seem quite unlikely that such an hypothetical potential contributes in an important way to the observations reported below.

B. The Potential Profile of the Toad Bladder with Ringer's Solution on Both Sides of the Membrane

THE SPONTANEOUSLY ACTIVE STATE A total of thirty-nine successful impalements were obtained in six bladders. The average transbladder potential was 54 mv, with a range of 26 to 81 mv. In every case, the initial stable potential change noted as the micropipette advanced into the bladder was a positive one. The first position beyond the mucosal surface of the bladder, presumably the interior of the bladder epithelial cell, was always found to be positive relative to the mucosal bathing medium. The size of this first potential step was quite variable, ranging from 4 mv in a preparation with a spontaneous transbladder potential of 27 mv to one of 44 mv in a bladder with a potential of 55 mv.

In evaluating the number of potential steps present in the bladder, it was essential to estimate the precision and reproducibility of the potential measurements. Experience with the experimental techniques and the preparation leads us to regard as significant only those changes in potential with alterations in position which exceed 10 per cent of the total transbladder potential. This limit of 10 per cent includes effects due to small changes in micropipette tip potential occurring during the course of an impalement, potential changes due to mechanical distortion of the micropipette, possible diffusion potentials across the serosal limiting membrane, and, in later experiments, errors in short-circuiting the bladder due to alterations in the condition of the preparation during an impalement or inaccuracies in the establishment of the short-circuited condition.

Given this limitation, in thirty-two of the thirty-nine impalements, more than 90 per cent of the total transbladder potential was accounted for by two potential steps within the bladder, both steps consisting of increasing positivity at the micropipette tip as it was advanced from the mucosal side of the bladder. In the remaining seven instances, three potential steps were noted, each of increasing positivity as the micropipette was advance from the mucosal medium.

THE CHRONICALLY SHORT-CIRCUITED STATE Since much of the information on the active transport of sodium in this preparation has been obtained

under conditions in which the spontaneous transbladder potential has been nullified by the passage of current from an external circuit, it seemed advisable to examine the potential profile under these circumstances as well. The bladders were set up with Ringer's solution on both sides of the bladder wall as before, but were maintained in the short-circuited state for from one-half to several hours before being impaled. At infrequent intervals during the course of an experiment, the spontaneous transbladder potential was checked by briefly switching off the short-circuit current.

A total of twenty-eight successful impalements was obtained in four bladders with a mean spontaneous transbladder potential of 51 mv and a range of 31 to 63 mv. The first stable potential recorded after contact with the mucosal surface of the bladder, presumably referrable to the interior of the bladder epithelial cells, averaged -8 ± 4 (S.D.) mv, and was always negative to the mucosal medium. Correction for the anticipated change in the micropipette tip potential on contact with the cytoplasm brings this figure to -5.5 mv.

THE INTERMITTENTLY SHORT-CIRCUITED STATE In those cell types for which the information is available, the resistance of the cell membrane is high relative to that of the cytoplasm. From these findings, it is reasonable to suppose that, excluding the low resistance serosal limiting membrane, the major resistance steps in the toad bladder will correspond to the plasma membranes of the mucosal and serosal surfaces of the bladder epithelial cells. The technique of intermittent short-circuiting while the micropipette is being advanced through the bladder gives information about the relative bladder resistance ahead of the micropipette tip, hence permits some conclusion as to the location of the pipette tip within the preparation.

Fig. 2 is the record of one of eleven impalements conducted under conditions of intermittent short-circuiting. The lower envelope of the curve shows the potential at the tip of the micropipette when the bladder is in the spontaneously active state, as a function of distance through the bladder, the reference electrode being on the serosal side. Two potential steps are seen, each positive relative to the mucosal medium; the potential after the second step is not significantly different from that of the serosal reference electrode. The upper envelope of the curve shows the potential profile of the bladder in the short-circuited state. Here there is no significant change in the potential at the electrode tip during its advance.

Since the short-circuit current is held constant during any one impalement, the change in the potential registered at the micropipette tip as the short-circuit current is switched on and off gives a value for the relative D.C. resistance of the portion of the bladder which lies ahead of the tip. The observation that the resistance is approximately halved after the first potential step indicates that the tip has penetrated into the bladder and that the potential

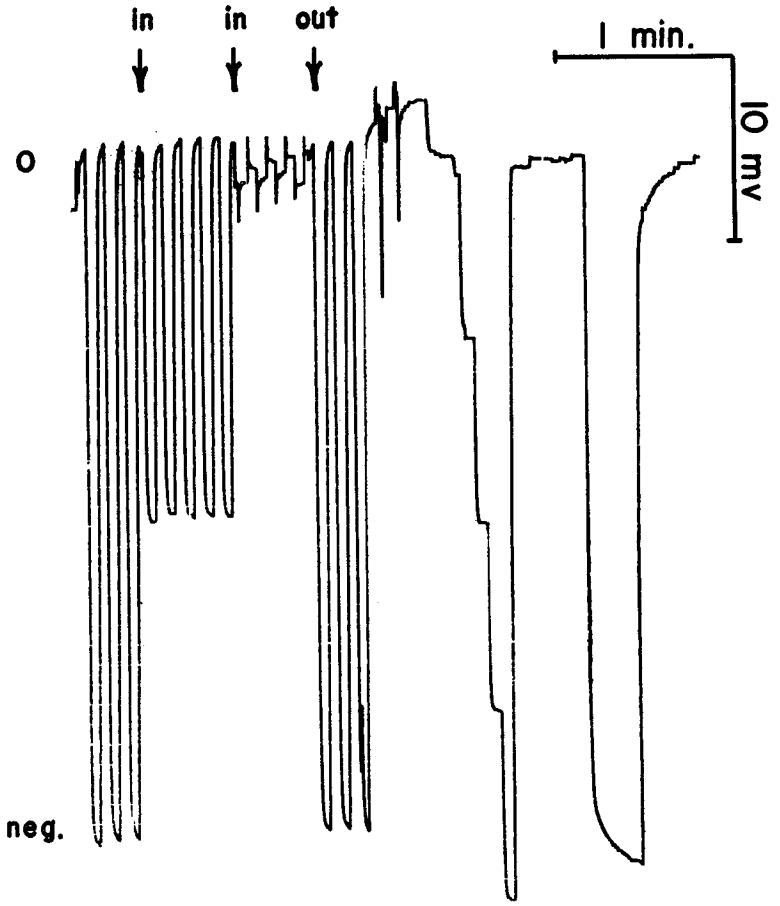

FIGURE 2. Record of the impalement of an intermittently short-circuited toad bladder with ordinary Ringer's solution on both sides of the membrane. The record is run continuously during the course of the impalement and withdrawal. The lower envelope of the curve initially shows the total transbladder potential with the micropipette in the mucosal medium. The first advance of the micropipette is associated with a positive potential step, the second advance with another step of the same polarity, the sum of the two equaling the total transbladder potential. The upper envelope of the curve indicates the potential profile of the bladder in the short-circuited state. The height of the vertical excursion of the record is proportional to the D.C. resistance of that portion of the bladder between the micropipette tip and the serosal reference electrode. Voltage calibration and spontaneous transbladder potential are shown on the right.

at some point beyond the mucosal membrane surface is being measured. The fact that half the relative bladder resistance remains ahead of the micropipette indicates that the tip has not passed on into the serosal connective tissue, hence must be in the interior of the bladder epithelial cell. The record is consistent with the observations in spontaneously active bladders, and further shows

that there is little or no potential change across the mucosal boundary in the short-circuited state, in accord with the results in chronically short-circuited preparations.

It is appreciated that, in general, the impedance of cell membranes is not independent of the potential across the membrane, hence it is customary to keep the applied electrical perturbation used to measure the impedance as small as the range of the measuring instruments permits. Here the short-circuit current was used, a disturbance of the order of 5 to 20 microamperes per cm^2. The additional information sought was the potential profile in both the spontaneously active and short-circuited states, of the same cell, during the same impalement. The cost of this information is that the calculated D.C. resistance becomes a chord of the current-voltage relation for the membrane rather than the measure of its slope at a point. In view of the fact that only an approximation of the membrane resistance was required to indicate the position of the micropipette tip, and that knowledge of other parameters, such as the electrical characteristics of the lateral walls of the bladder epithelial cells and their relation to the extracellular space, was lacking, it seemed inappropriate to attempt a more complete and precise analysis of changes in bladder impedance during impalement.

Although the switching rate of nine per minute was slow relative to the purely electrical time constant of the membrane, it might not be slow relative to the time constant for the readjustment of the ionic composition of the cell water after an applied potential. If true, this circumstance might alter the shape of the potential profile of the bladder measured during intermittent short-circuiting, making invalid the extension of the relation of potential and position to bladders in the chronically short-circuited or spontaneously active states. The observed similarities between the potential profiles of bladders in either of these two steady states and those of the intermittently short-circuited preparations indicate that the non-steady state condition does not alter the potential profile significantly and support the validity of the correlation between position and potential obtained from the latter type of study.

Mention has been made previously of potential profiles which showed more than the usual two steps. An example of such an impalement is shown in Fig. 3. The possibility that such a finding in the spontaneously active bladder could be due to mechanical distortion of the micropipette with resultant changes in the registered potential is made unlikely by the observation that the changes in potential are associated with changes in the relative bladder resistance ahead of the micropipette. A change in recorded potential is associated with a change in the electrically determined position of the tip. The record also shows only a modest negative potential step in the short-circuited state as the micropipette crosses the mucosal boundary, in support of the findings in chronically short-circuited preparations.

These observations of more than two potential steps in the electrical profile of the toad bladder are in the minority, but their significance is not yet clear. Electron microscope studies of the toad bladder do not suggest continuous intracellular structures parallel to the bladder surface which might furnish

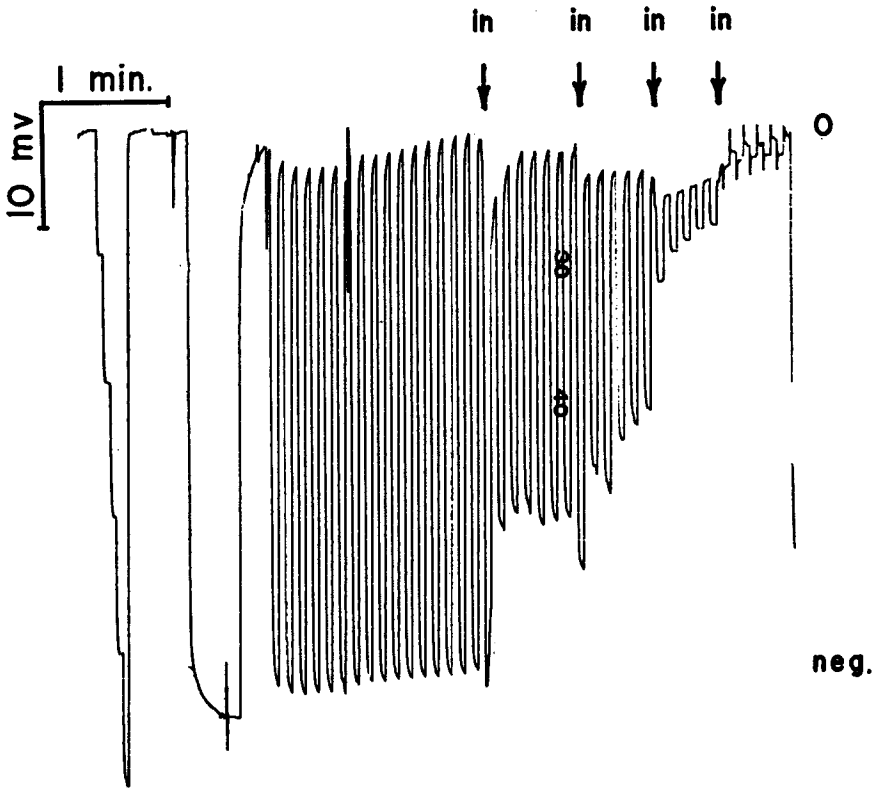

FIGURE 3. Record of the impalement of an intermittently short-circuited toad bladder with ordinary Ringer's solution on both sides of the preparation. The record illustrates the potential profile in a minority of cases in which more than two potential steps were noted during the passage of the micropipette through the membrane. See legend of Fig. 2.

The numerals in bold face type overlying the potential tracing in this and the following figure are printed on the chart paper and are unrelated to the potential measurement.

additional barriers to the movement of ions (6). The bladders were not stretched tightly over the Ringer-agar pillar in mounting, so that it is conceivable that the micropipette might enter the bladder through the mucosal surface of one cell and pass across the lateral borders of one or two others before finally entering the serosal medium. The absolute values of the cellular potentials in a given bladder are variable enough so that such a path would give rise to a series of potential steps.

The present evidence strongly supports a simple two step potential profile in the majority of cells on the musocal surface, and does not permit of a unique explanation for the observation of more than two steps in some impalements.

C. The Potential Profile of the Toad Bladder with Low Sodium Concentration on the Mucosal Side and Ringer's Solution on the Serosal Side of the Membrane

The isolated toad bladder is capable of active transport of sodium even from mucosal media of very low sodium concentration (7). If electrical forces are important in the net entry of sodium across the mucosal boundary, a significant negative potential step must appear at the mucosal surface as the concentration of sodium in the mucosal medium is reduced to low levels.

Changes in the concentration of sodium in the mucosal media were achieved by the dilution of the Ringer's solution with appropriate volumes of a solution in which choline was substituted for sodium. The potential profile of the toad bladder was examined at concentrations of sodium in the mucosal medium of 60, 6 to 10, and under 1 meq per liter. With the exception of a decrease in the total transbladder potential and short-circuit current with decreasing concentrations of sodium, the results of the studies, including the transmucosal potential gradient in the short-circuited state, were similar and only those experiments will be cited in which the mucosal concentration of sodium was under 1 meq per liter.

THE CHRONICALLY SHORT-CIRCUITED STATE As outlined in a previous section, the bladders were maintained in the short-circuited condition for more than one-half hour before the impalements were carried out. A total of thirteen successful impalements in three bladders was obtained in which the concentration of sodium in the mucosal solution, measured at the end of the experiments, was less than 1 meq per liter. The mean spontaneous transbladder potential, determined during brief open-circuiting, was 12 mv, with a range of 7 to 16 mv. The mean potential change during impalement was -6 ± 5 mv (S.D.), with the intracellular potential negative to the mucosal medium. Correction for the estimated change in the tip potential of the micropipette would alter this figure to -3.5 mv. The intracellular location of the micropipette tip was checked once or twice during the course of an impalement by very briefly switching off the short-circuit current and comparing the change in the observed potential with the similarly determined spontaneous transbladder potential. The fractional D. C. resistance of that portion of the bladder ahead of the micropipette tip at the time when the "intracellular" potentials were recorded was 0.5 ± 0.1 (S.D.), indicating that the tip was in the substance of the bladder.

THE INTERMITTENTLY SHORT-CIRCUITED STATE Fig. 4 is the record of an impalement in an intermittently short-circuited bladder bathed on its mucosal surface by a solution containing 0.26 meq per liter of sodium. The tracing demonstrates the low spontaneous transbladder potential associated with low concentrations of sodium in the mucosal medium, and confirms the small size of the negative potential step on crossing the mucosal boundary which was

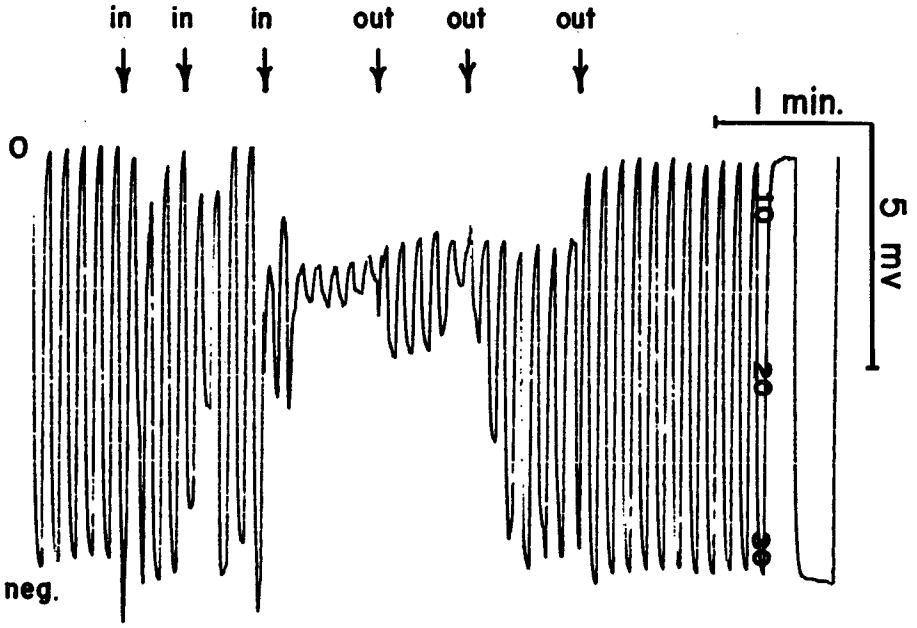

FIGURE 4. Record of the impalement of an intermittently short-circuited toad bladder with choline-Ringer's solution (Na = 0.26 meq per liter) on the mucosal side and ordinary Ringer's solution on the serosal side. The record demonstrates the decreased value of transbladder potential seen with mucosal solutions of low sodium concentration. In the spontaneously active state, the interior of the bladder is at the same potential as the mucosal medium or positive to it. In the short-circuited state, the bladder interior is slightly negative to the mucosal medium. See legend of Fig. 2.

noted in the chronically short-circuited preparations. It further shows the presence of the expected potential profile containing at least two positive potential steps on going from mucosal to serosal medium in the spontaneously active state.

DISCUSSION

Koefoed-Johnsen and Ussing (8) have formulated a simple and ingenious descriptive hypothesis to account for the origin of the electrical potential of

frog skin, a tissue which the toad bladder resembles in several important respects. In terms appropriate to the toad bladder, the model assumes that the mucosal surface is selectively permeable to sodium, which enters the cell passively, along its concentration gradient. The serosal surface is regarded as being selectively permeable to potassium, which diffuses out of the cell into the serosal medium down its concentration gradient. The concentration gradients for sodium and potassium are maintained by an active transport mechanism at the serosal boundary which extrudes sodium from the cell into the serosal medium and takes up potassium from the serosal medium and transports it into the cell.

It is a consequence of the model that, in the spontaneously active state, there should be a positive-going potential step on passing from the mucosal medium into the cell, and another of the same polarity on leaving the cell at the serosal border. Although the initial study by Ottoson *et al.* (9) demonstrated only one potential step in the frog skin, subsequent micropipette studies by Engbaek and Hoshiko (10) and by Scheer and Mumbach (11) have confirmed the presence of the anticipated two step potential profile. The findings in the spontaneously active toad bladder reported here also show two potential steps of the expected polarity in the majority of impalements and are consistent with the Ussing hypothesis.

This study was undertaken primarily to investigate the forces responsible for the net entry of sodium into the toad bladder from mucosal solutions of low sodium concentration, the situation in which the bladder normally finds itself *in vivo*. The demonstration of a positive potential step across the mucosal boundary in the spontaneously active state, even when the mucosal concentration of sodium is less than 1 meq per liter, means that electrical driving forces cannot play a role in bringing about net entry of sodium. In the short-circuited state a small negative potential step is present, favoring the net entry of sodium. The available information is not sufficient to permit quantitative evaluation of the contribution this driving force of about 5 mv makes to the observed net transport of sodium, but it seems unlikely that its role is a very large one in situations in which mucosal concentration of sodium is high. When the concentration of sodium in the mucosal fluid is low, even this small electrical driving force may be an important part of the total driving force for net sodium entry.

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